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# The leaf color performance on several lines of cassava and its relation with tuber yield as early reference

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**Abstract**

Mutation breeding using Gamma irradiation is one of strategies to improve the genetic variability in a vegetatively propagated plant such as cassava. Characterization of the qualitative and quantitative characters of irradiated cassava mutant lines is an important activity in the selection process. Leaf color variability can be used to estimate the potential mutant genotypes without waiting the tuber harvest time using hyperspectral technology, and thus accelerate the plant breeding program. The objectives of this research were to characterize leaf qualitative characteristics and yield characteristics of several irradiated cassava M1V2 generation and to evaluate the relation between leaf performance and tuber yield of several cassava mutant lines as an early reference in the hyperspectral remote sensing application. Fifteen genotypes of M1V1 generation (five parent genotypes and ten mutant lines derived from gamma rays irradiation) were planted in the experimental field. Leaf performance such as color of leaf (young shoot and mature), petiole, and also other characters were observed by using cassava descriptor. The result showed that the leaf and plant canopy performance play an important role in determining the yield (weight of fresh tuber). In particular genotypes, greener (dark green) leaf color will increase the weight of tubers per plant. These results confirmed that the leaf color of several cassava lines would be well suited for early estimation of yield.


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*Keywords:*irradiation; mutant lines; qualitative characters; M1V1; M1V2

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## 1. Introduction

Cassava is the third staple food in Indonesia after rice and maize. Cassava is used as food, feed, and wide variety of industrial raw materials. Cassava is also one of the potential commodities that are able to provide the raw material of bioethanol [1]. Efforts in cassava development are still constrained by the low production, thus creating new varieties of cassava with high yield is really necessary.

Conventional breeding of cassava face some limitations, because of high ploidy level, high heterozygosity, the presence of inbreeding depression, and low genetic variability due to the conventional multiplication through plant vegetative parts [2, 3]. One of the strategies to increase the genetic variability of cassava is using mutation induction. Mutation induction can be done by using chemical mutagens or physical mutagens. However, the mutation induction by irradiation on the vegetative parts of plants gives better results when compared with the chemical mutagen induction. This is due to the low absorption capacity of the plant vegetative tissues and the possibility of chemical liquid residue left on the plant tissues that used for food material. Therefore, the use of physical mutagens such as Gamma-ray irradiation would the better choice.

Along with the increasing demand of cassava, the putative mutants obtained from the Gamma-ray irradiation is expected to be potential candidates for the new high yield cassava varieties. Cassava harvesting time becomes one of the constraints in cassava development, because these plants need 9-12 months before harvest [4]. Therefore, an appropriate technology in estimating the productivity of cassava to support the fulfillment of domestic demand is needed.

Remote sensing technology can be utilized for the model development to estimate some food crops productivity, such as rice [5], and detect the biotic and abiotic stresses of plants [6]. Hyperspectral remote sensing technology is a remote sensing which utilizing the hyper number of channel so the user will get wider opportunities to develop applications according to the needs, especially in the context of natural resource management and the environment. This is not found in the multispectral system which used limited number of channels. Hyperspectral technology is a method to obtain a picture of the Earth's surface simultaneously by hyper number of bands / channels (more than 200) and using a narrow wavelength (narrow band) and adjacent [7].

The data of qualitative and quantitative character from cassava mutants, such as leaf and petiole color, and its relationship to the production variable can be used as the baseline for predicting the hyperspectral data model. Hyperspectral data can provide accurate information about the plant condition in an area that can be distinguished, such as observation of plant growth phase, crop biophysical parameters, pests and plant diseases and the productivity of the crop [8].

The objective of this research were to characterize leaf qualitative characters and yield characters of several irradiated cassava namely Jame-jame, Ratim, UJ-5, Malang-4, and Adira-4 at M1V1 and M1V2 generation and to evaluate the relation between leaf performance and tuber yield of several cassava elite lines as the first spectral reference on hyperspectral remote sensing applications as well as computational models on photogrammetry techniques.

## 2. Materials and Method

The research was conducted in Cikabayan experimental field, Bogor Agricultural University (240 m asl) in June 2012 - September 2013. Gamma ray irradiation treatment was applied in the Laboratory of Isotope Technology Application Center and Irradiation (PATIR), National Nuclear Energy Agency (BATAN), South Jakarta, Indonesia. The planting materials used were eight to ten months cassava cuttings, with five buds.

The research was arranged in a randomized complete block design (RCBD) with two factors. The first factor was the cassava genotypes, i.e. Jame-jame (v1), Ratim (v2), UJ-5 (v3), Malang-4 (v4), and Adira-4 (v5). The second factor was the irradiation dose consisted of five levels, i.e. 0 (d0), 15 (d1), 30 (d2), 45 (d3), and 60 Gy (d4). There were 25 treatment combinations and each combination consisted of three blocks (the position of cassava cuttings). The position of cassava cuttings used were the top section (30% of the stems), the middle section (40% of the stems), and the basal section (30% of the stems). Each block consisted of five stem cuttings, thus there were 375 experimental units.

Five buds cassava cuttings were treated with Gamma ray irradiation in the PATIR BATAN Laboratory using a Gamma Chamber 4000A based on the treatment dose, except the control treatment (0 Gy). Irradiated cassava cuttings were planted in the Cikabayan experimental field, with 1 m x 1 m plant spacing. Cassava plants were fertilized with urea, SP-36, and KCl with the rate of 200, 150, and 150 kg $ha^{-1}$, respectively. SP-36 fertilizer was given entirely at planting, Urea was given 1/3 of the rate at planting and 2/3 of the rate at the age of one month after planting (MAP), whereas KCl was given at 2 MAP. The weed was manually controlled every 2-3 weeks. Soil ridging was conducted in conjunction with the second application of Urea fertilizer.

The characterization was done at 6 MAP and at harvest time (11 MAP). The observation was carried out by scoring qualitative character of the young leaves color (unfolded leaf), mature leaf color, and the petiole color based on PPVT characterization guideline [9] and the type of tubers neck, tuber shape, and tuber flavor based on IITA characterization guideline [10]. The quantitative character observations were conducted on the weight of tubers per plant, number of tubers per plant, and number of commercial tuber (tuber length > 20 cm) per plant based on IITA [10].

## 3. Results and Discussion

The Based on the observation of leaves qualitative character on the plants of M1V1 generation, it appears that these five parent genotypes have a phenotypic variability. Some mutant plants showed phenotypic variability compared to the parent genotype on the number of lobes, lobe size, mature leaf color, leaf vein color, and petiole color. The leaves of mutant plants from Jame-jame, Ratim, UJ-5, and Adira-4 genotype had 7 lobes, while Malang-4 genotype had 8-9 lobes (Figure 1). Red leaf vein was only owned by mutants from Jame-jame and Ratim genotype, while other mutant genotypes had green leaf vein. The leaf color of all mutant genotypes was green, but mutants from UJ-5 genotype had a slightly pale green color. Mature leaf lobe size (ratio of length: width lobe) on mutants from Jame-jame, Ratim, and Malang-4 was relatively equal, while mutants from UJ-5 had larger lobe length, and mutants from Adira-4 had narrower lobes width. Petiole color of mutants from Jame-jame and Ratim was red along the petiole, UJ-5 and Adira-4 mutants had green petiole color with red color near the leaf and Malang-4 mutants had green color along the petiole. Phenotypic variability of cassava mutants especially on leaf qualitative characters is shown on Figure 1.

Jame-jame Ratim UJ-5 Malang-4 Adira-4

Fig. 1. Variability on cassava leaf characters (number and size of lobe, leaf color, and petiole color) of five parent genotypes

Gamma ray irradiation treatment did not only change the cassava plant phenotype but also increased the variability on yield characters (tuber weight per plant and number of economic tubers per plant). These changes can be seen on several qualitative characters in several cassava mutants of M1V1 generation genotypes (Table 1). Jame-jame mutant genotypes from 15 and 30 Gy irradiation dose had higher tuber weight per plant than the parent genotype. The same result was also shown by Malang-4 genotypes, where mutant from 15 and 30 Gy irradiation dose had higher tuber weight per plant than the parent genotype. Increasing number of tubers only appears on Malang-4 and Adira-4 mutants with 15 Gy irradiation dose, whereas number of economic tubers which higher than the parent genotype only on mutant from Malang-4 genotype.

Table 1. Yield characters on several cassava mutant M1V1 generations

| Genotype | Gamma ray irradiation dose (Gy) | Tuber weight per plant (kg) | Number of Tuber per plant | Number of economic tuber per plant |
|---|---|---|---|---|
| Jame-jame | 0 | 7.18 ± 0.76 | 10.13 ± 1.75 | 8.00 ± 1.40 |
| | 15 | 9.25 ± 2.29 | 7.33 ± 1.66 | 6.17 ± 1.38 |
| | 30 | **10.90 ± 7.72** | 7.56 ± 5.09 | 7.11 ± 4.62 |
| Ratim | 0 | 10.27 ± 1.33 | 12.42 ± 1.42 | 9.50 ± 1.64 |
| | 15 | 8.62 ± 3.55 | 9.65 ± 3.43 | 7.40 ± 2.95 |
| | 30 | 3.57 ± 3.61 | 4.67 ± 2.52 | 1.33 ± 1.15 |
| UJ-5 | 0 | 10.84 ± 2.32 | 14.08 ± 1.65 | 10.40 ± 0.53 |
| | 15 | 9.12 ± 4.11 | 9.30 ± 3.50 | 6.43 ± 2.69 |
| | 30 | 4.56 ± 1.59 | 7.06 ± 1.83 | 4.03 ± 0.46 |
| Malang-4 | 0 | 8.63 ± 0.25 | 9.50 ± 2.05 | 7.85 ± 1.13 |
| | 15 | **14.07 ± 1.53** | **11.45 ± 1.34** | **9.60 ± 0.53** |
| | 30 | 11.82 ± 1.82 | 8.00 ± 1.00 | 7.33 ± 1.15 |
| Adira-4 | 0 | 13.73 ± 2.31 | 10.64 ± 0.92 | 7.87 ± 0.23 |
| | 15 | 10.70 ± 2.49 | **11.02 ± 2.85** | 7.96 ± 1.97 |
| | 30 | 9.82 ± 5.73 | 7.17 ± 1.26 | 5.75 ± 1.75 |
| Standard Error | | 0.625 | 0.462 | 0.408 |

Applications of Gamma ray irradiation also improved the variability of several tuber characters such as tuber shape, tuber neck type, and tuber taste on several cassava genotypes (Table 2). Gamma irradiation changed the tuber neck type of UJ-5 and Malang-4 from sessile to mixed and penduncúlate, respectively. Mutants generated from Ratim genotype also showed variability in the tuber shape, i.e. cylindrical, cylindrical-conical, and irregular tuber shape, while the parent genotype has cylindrical tuber shape. Tuber shape of mutants generated from UJ-5 and Malang-4 genotypes was dominated with cylindrical shape. The taste of tuber of Ratim genotype was sweet, while tubers of mutants generated from this genotype had sweet and intermediate taste. Gamma irradiation changed the tuber taste of several mutants generated from UJ-5 and Malang-4 genotypes from bitter (parent genotype) to intermediate and sweet.

Plant breeding program often use indirect approach to improve the plant characters, such as yield character. One of the methods is selecting several characters that related to the yield character. Two or more characters that have the positive correlation will make the selection process easier. Otherwise, the negative correlation would make the process become difficult to get the expected characters. If there is no correlation found between the expected characters, the selection process will become not effective [11].

The petiole length character have a significant positive correlation with the lobe length, lobe width, and lobe size on mature leaf (ratio of length: width on leaf lobe) (Table 3). The longer petiole length would increase the lobe length and width and also the lobe size of mature leaf. Petiole length character also had a significant positive correlation with number of tubers per plant and number of economic tubers per plant characters. This indicated that an increase on petiole length could increase number of tubers and number of economic tubers.

Table 2. Variability of cassava mutant at M1V1 generation on tuber neck type, tuber shape, and tuber taste

| Character | Genotype | Parent genotype | M1V1generation mutant |
|---|---|---|---|
| | Ratim | sessile | Mixed |
| Tuber neck type | UJ-5 | sessile | Mixed and pendunculate |
| | Malang-4 | sessile | Sessile and mixed |
| | Ratim | cylindrical | Conical-cylindrical, cylindrical, irregular |
| Tuber shape | UJ-5 | conical | Dominant cylindrical |
| | Malang-4 | conical- cylindrical | Dominant cylindrical |
| | Ratim | Sweet | Sweet to intermediate |
| Tuber taste | UJ-5 | Bitter | Intermediate to bitter |
| | Malang-4 | Bitter | Sweet to bitter |

The tuber weight character had a significant positive correlation with the stem diameter. Increase in stem diameter would increase tuber weight per plant. Tuber weight character also had a significant positive correlation with number of tubers per plant and number of economic tubers per plant, which increase in number of tubers per plant and number of economic tubers characters would increase tuber weight per plant. The same result was also reported by Sundari *et al*. [12] that number of tubers per plant character and tuber diameter on each cassava genotypes showed a significant positive correlation with the tuber yield. The positive correlation found on harvesting characters, such as stem diameter, number of tuber per plant, and number of economic tuber per plant, indicated that those three characters could be used for estimating the cassava yield potential (tuber weight).

Table 3. Correlation score between several quantitative characters on cassava mutant M1V1 generation

| | PL | LL | LW | LS | CT | NT | NET | SD | TW |
|---|---|---|---|---|---|---|---|---|---|
| PL | | 0.957** | 0.952** | 0.888** | -0.091 | 0.215* | 0.339** | -0.047 | 0.097 |
| LL | | | 0.961** | 0.936** | -0.068 | 0.195* | 0.312** | -0.006 | 0.082 |
| LW | | | | 0.847** | -0.061 | 0.215* | 0.338** | -0.019 | 0.102 |
| LS | | | | | -0.086 | 0.161 | 0.262** | -0.013 | 0.064 |
| CT | | | | | | 0.005 | -0.106 | 0.150 | 0.166 |
| NT | | | | | | | 0.821** | 0.159 | 0.619** |
| NET | | | | | | | | 0.087 | 0.675** |
| SD | | | | | | | | | 0.376** |
| TW | | | | | | | | | |

*significant correlation on α=5% ; **significant correlation on α=1%. PL = petiole length, LL = lobe length, LW = lobe width, LS = lobe size on mature leaf, CT = cortex thickness, NT = number of tuber per plant, NET = number of economic tuber per plant, SD= stem diameter, TW = tuber weight per plant

Stem cuttings from M1V1 population that already harvested were immediately separated and stored in a shaded place. The stem cuttings were then planted on the field that already plowed to get the M1V2 generation. Observation on cassava mutant at M1V2 generation and the parent genotype was done for several characters such as leaf qualitative characters. The observation becomes more interesting because the changes on cassava mutant leaf qualitative character must be compared with the parent genotype to observe their stability.

The observation result on cassava mutant leaf qualitative character, especially on mature and apical leaf color, and the leaf petiole showed the variability when compared with the parent genotype. Gamma ray irradiation changed the apical leaf color, petiole color, and mature leaf color of the mutant lines. The variability on leaf qualitative character is presented on Figure 2.

Apical leaf color on Ratim and UJ-5 and the mutants from irradiation dose of 15 Gy and 30 Gy looks more stable, which was purplish green and mutants from Malang-4 genotype had green color. The apical leaf color of mutants generated from Jame-jame and Adira-4 genotypes were changed into light green and green. Mature leaf color of several mutants generated from UJ-5, Adira-4, and Malang-4 was changed into dark green, which was darker than the mature leaf color of the parent genotypes. Mature leaf color on Jame-jame and Ratim mutants were apparently stable.

0 Gy 15 Gy 30 Gy

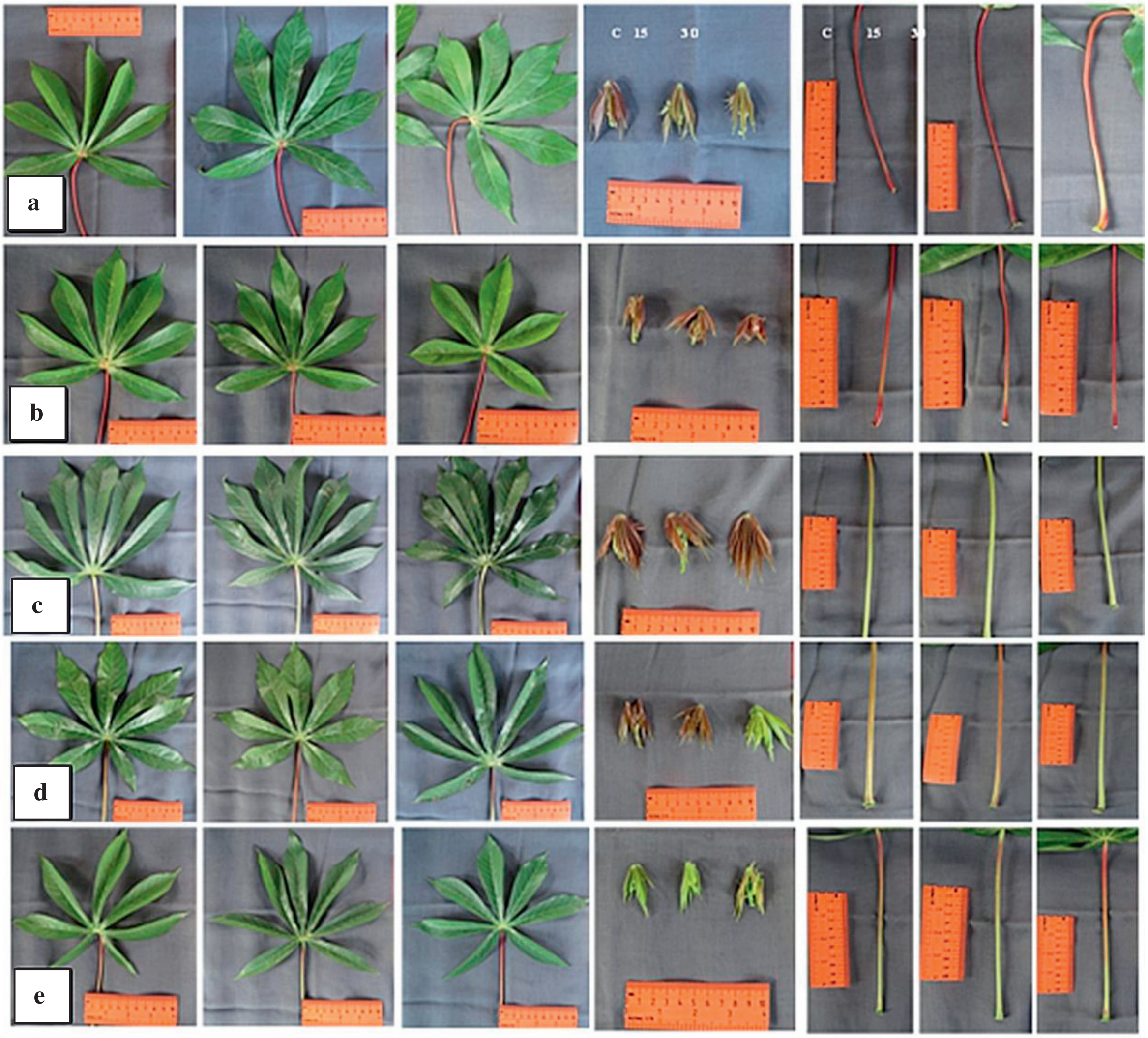


Fig 2. Leaf characters of cassava mutant lines. a) Jame-jame, b) Ratim, c) UJ5, d) Adira-4, and e) Malang-4

The mutation effects on the cassava stem cuttings with several irradiation doses were random and individuals, including the result of mutation effect on stem cuttings with the same dose application. The mutation effect showed on Figure 2, which the 15 Gy dose change the petiole color become greenish red and green. Plant breeding program through mutation method have the lack side, such as random effect of mutation and cannot focused on specific gen so the breeder can’t predicting the result of the mutation [13]. Furthermore, irradiation dose of 45 Gy suspected could harm the plant cells and occur diplointic selection, where mutant cells are oppressed with the normal cells, so the plant tuber have a lower weight than the average parent genotype tuber weight.

The results of this research show the color variability in mutant plants and the presence of correlation between the leaves color with the tuber yield variable provide an opportunity to get potential mutant genotypes in the population through the hyperspectral remote sensing application. These estimation results can accelerate cassava breeding activity, because cassava has a long harvest time (8-12 months).

Hyperspectral remote sensing is not only used for plant characterization but also their health status. In contrast to conventional point measurements, imaging detects the distribution and quantity of signals and thus improves the interpretation of fluorescence and reflectance signatures. In multispectral fluorescence and reflectance set-ups, images are separately acquired for the fluorescence in the blue, green, red, and far red, as well as for the reflectance in the green and in the near infrared regions. In addition, 'reference' color images are taken with an RGB (red, green, blue) camera. Examples of imaging for the detection of photosynthetic activity, UV screening caused by UV-absorbing substances, fruit quality, leaf tissue structure, and disease symptoms are introduced [14].

Hyperspectral remote sensing is very useful in cassava breeding research. The hyperspectral technology could estimate the potential mutant genotypes using leaf color variability without waiting the tuber harvest time, and thus accelerate the plant breeding program. Color of leaves on mutant plants that estimated by the picture also can be used to estimate the nutrient deficiency suffered by plants and the condition of the plant pathogen infections or diseases that occur on the plants. Preliminary results of this research are very useful to continue the study of these mutant cassava genotypes to obtain plant stability and plant yield information in a faster time.

## 4. Conclusion

Gamma ray irradiation caused morphological mutations and variability on leaf and tuber yield characters on cassava mutants at M1V1 generation. Darker green leaf color showed a significant positive correlation with tuber weight, thus several mutant lines with darker leaf color had higher tuber weight than the parent genotype. Changes on leaf qualitative character still appear on mutant plants at M1V2 generation. The use of hyperspectral remote sensing could estimate the high tuber yield character on cassava mutant's plant population by the color of leaves. The hyperspectral technology could estimate the potential mutant genotypes without waiting the tuber harvest time.

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